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# The effect of human antibacterial peptide LL-37 in the pathogenesis of chronic obstructive pulmonary disease

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## KEYWORDS

Chronic obstructive  
pulmonary disease;  
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## Summary

**Background:** Previous research has shown that innate immune system was more important than the acquired immune system in the pathogenesis of COPD. LL-37 is the only human cathelicidin identified so far. As an integral part of the innate immune system, besides antibacterial activity, its chemotactic activity, damage repairing, influencing apoptosis and its cytotoxicity are attracting people's attention. The aim of the present study was to evaluate role of LL-37 in the pathogenesis of COPD.

**Methods:** ELISA and immunohistochemistry were applied to investigate the expression of LL-37 in induced sputum and lung tissue of COPD patients. Bronchial epithelial cell (BEP2D) and alveolar epithelial cell (A549) were treated with LL-37 synthesis polypeptide in vitro to assess the role of LL-37 in inflammation and apoptosis.

**Results:** We found that increased induced sputum levels of LL-37 in COPD patients were associated with airflow limitation, health status and exercise tolerance and the expressing intensity of LL-37 in both airway district and pulmonary alveoli area in COPD group significantly increased compared with control group. Through stimulation by CSE and LPS, the expression of LL-37 was increased in bronchial epithelial cell and alveolar epithelial cell. LL-37 synthesis polypeptide can promote the releasing of inflammatory factor IL-8 and induce apoptosis of bronchial epithelial cell and alveolar epithelial cell.

**Conclusion:** This study suggested that LL-37 may play important role in the pathogenesis of COPD and may be a possible novel therapeutic target in COPD.

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Chronic obstructive pulmonary disease is one of the leading causes of morbidity and mortality worldwide, and its prevalence is increasing. Progressive airflow limitation and symptoms in COPD are associated with an abnormal inflammatory response of the lung to noxious particles or gases.<sup>1</sup>

The innate immune system of the lung protects against infection and regulates inflammatory responses and adaptive immunity. Various studies have highlighted the role of the innate immune system in COPD.<sup>2,3</sup> Antimicrobial peptides (AMPs) are now considered as an essential part of innate immunity because of their ability of killing invading respiratory pathogens and their various other activities. The defensins and cathelicidins are the principal components of AMPs families. LL-37 is the only human cathelicidin identified so far.<sup>4</sup> As an integral part of the innate immune system, this protein is involved in the first line of host defense. LL-37 displays antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi and viruses, neutralizes LPS bioactivity, and so protects against endotoxic shock.<sup>5,6</sup> Besides antibacterial activity, its chemotactic activity, damage repairing, influencing apoptosis and its cytotoxicity are attracting people's attention.<sup>7–13</sup> Chronic inflammation and airway remodeling are properly two main pathological processes in the pathogenesis of COPD. Previous studies of our team showed that LL-37 is elevated in induced sputum in COPD patients and inversely related to lung function.<sup>14,15</sup> We presume that with the wide spectrum and multi-effect biological function, LL-37 may play important role in the pathogenesis of COPD.

In this study, we thus examined the expression of LL-37 in induced sputum and lung tissue of COPD patients and assessed the possible role of LL-37 in inflammation and apoptosis.

## Patients and methods

### Patients and controls

A total of 60 subjects, including 28 stable COPD patients, 14 healthy current smokers and 18 healthy non-smokers were enrolled in this study. The lung tissues from 9 COPD patients and 10 controls receiving operation because of lung cancer, lung bullae, lung cyst from an uninvolved segment of the subpleural parenchyma at least 5 cm away from diseased region were collected for immunohistochemical staining. The diagnosis of these COPD patients was made according to the criteria of global initiative for chronic Obstructive Lung Disease (GOLD 2007). All patients were recruited from hospitalized or the Outpatients Department at Qilu Hospital, Shandong University, China. 14 healthy current smokers and 18 healthy non-smokers are health care workers of our hospital. All participants met the following criteria: no exacerbation, no use of inhaled or oral corticosteroids for 3 months prior to sample collection, and no respiratory tract infection for 1 month before the study. Patients with bronchiectasis, asthma, interstitial diseases and heart failure were excluded. An exacerbation was defined as the presence, for 2 days consecutively, of an increase in any two major symptoms (dyspnea, sputum

purulence and sputum volume) or in one major and one minor symptom (wheeze, sore throat, cough and symptoms of a common cold).<sup>16,17</sup> The study was approved by the Ethics Committee of Shandong University, and written informed consent was acquired from each individual.

### Cell lines and culture conditions

BEP2D (ATCC) was cultured at 37 °C in 5% CO<sub>2</sub> in LHC-8 (GIBCO, GrandIsland, NY). The AECs (A549 cells) were cultured in DMED supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL) at 37 °C in 5% CO<sub>2</sub>. The culture medium was changed every 2 days. Cells were passaged every 4–5 days with 0.25% trypsin (Sigma, USA).

### Reagents

Kentucky standard reference cigarettes 2R4F were used for cigarettes smoke extraction (CSE). The cigarettes were conditioned at 22 °C and 60% relative humidity for at least 48 h before use. JJD100 single channel smoking machine was used with parameter of once per 2 s and 1 min' intervals. Cigarette smoke was diluted in D-hanks and filtered through a 0.22 µm-pore filter to remove bacteria and stored in –80 °C.

Lipopolysaccharides (LPS) (Sigma, USA) was diluted in serum-free DMEM into 100 µg/mL and filtered through a 0.22-µm-pore filter to remove bacteria.

LL-37 polypeptide (amino acid sequence: LLGDFFRKSKE-KIGKEFKRIVQRIKDFLRNLPRTES) was synthesized by GL Biochem (Shanghai) Ltd in China.

### Pulmonary function, health status and exercise tolerance

Pulmonary function was measured using a spirometer (Jaeger, Hoechberg, Germany), according to standardized guidelines.<sup>18</sup> Measurements were always performed by the same technician using the same spirometer. Health status was assessed using the St. George's Respiratory Questionnaire (SGRQ).<sup>19</sup> Scores were based on a scale of 0–100, with lower scores indicating better health status. The 6MWD was used to evaluate patients' exercise tolerance. Subjects were given standardized instructions to walk the greatest distance possible in 6 min and were given verbal encouragement. The 6MWD test was performed according to the American Thoracic Society guidelines.<sup>20</sup>

### Collection and preparation of blood samples

Peripheral venous blood samples (5 mL) were taken and were centrifuged at 13,000 g for 15 min at 4 °C. Serum was aspirated and frozen at –80 °C for subsequent analysis.

### Sputum induction and processing

After measurement of pulmonary function, salbutamol was administered (200 mg by metered-dose inhaler), and 15 min later subjects inhaled hypertonic (4%) saline delivered by

an ultrasonic nebulizer (DeVilbiss 65; DeVilbiss Corporation, Somerset, PA, USA).<sup>21–24</sup> Subjects were encouraged to cough, and sputum was collected in clean polypropylene cups. The sputum specimen was examined within 2 h and the viscous portions were selected and weighed. A freshly prepared 1:10 dilution of dithiothreitol (DTT; Sputolysin; Calbiochem Corp., San Diego, CA, USA) in distilled water was added in a volume equal to four times the weight of the selected sputum specimen. Samples were placed in a shaking water bath at 37 °C for 15 min, then further diluted with PBS to a volume equal to the sputum plus DTT. The suspension was filtered through gauze to remove mucus, and centrifuged at 120 g for 8 min. The supernatant was aspirated and frozen at –80 °C for subsequent analysis. The cell pellet was suspended in a volume of PBS equal to that of the aspirated supernatant. The total cell count and viability (Trypan blue exclusion method) were determined using a hemocytometer. Only samples with cell viabilities >50% and <20% squamous cell contamination were included in the analysis.

### Measurement of LL-37 in serum and induced sputum

The concentrations of LL-37 in serum and induced sputum supernatants were assayed using commercial ELISA kits according to the manufacturers' instructions. (LL-37: Hycult Biotechnology, Uden, the Netherlands). All assays were performed in duplicate for each sample, and the mean values are reported.

### Cell viability test

Cell viability was measured by the MTT assay based on the protocol described previously. BEP2D and A549 were seeded in 96-well culture plate at a density of  $1 \times 10^4$  cells/well in a final volume of 100  $\mu$ l medium. Approximately 24 h after seeding, CSE and LPS were added to the well at different concentrations for 6, 12, 24, 48 h. After treatment, culture medium was removed and 10  $\mu$ g of MTT (0.5 mg/ml) dissolved in culture medium was added and incubated for 4 h at 37 °C. 100  $\mu$ l dimethyl sulfoxide (DMSO) was added to each well. Plates were gently shaken for 10 min and the absorbance was recorded at 560 nm using a microplate spectrophotometer (BIO-RAD Mode 680, CA, USA). The cell viability was expressed as the percentage of absorbance of treated group to untreated control group.

### Immunohistochemical staining and double immunofluorescence staining

LL-37 in human lung tissues was examined by immunohistochemistry. Formalin fixed paraffin-embedded lung tissue sections were stained with rabbit anti-human LL-37 (Abcam, USA) and biotinylated goat anti-rabbit Ig (Maixin Co., Fuzhou, China) followed by streptavidin-conjugated peroxidase. DAB was used as substrate. Positive cells were counted in 50 different fields by 2 independent observers. For double immunofluorescence staining, BEP2D and A549 were incubated with anti-LL-37-FITC and DAPI for 60 min at

room temperature successively and then determined by laser scanning confocal microscope. Immunostaining was evaluated with the guidance of a pathologist who did not have access to the study. The mean of the data from two different observers was calculated.

### Measurement of IL-8 and TNF- $\alpha$ levels in cell culture supernatants

BEP2D and A549 cells were cultured with serum-free DMEM containing different concentration LL-37 polypeptide for 24 h. Then the supernatants were harvested. IL-8 and TNF- $\alpha$  were quantified by ELISA kits supplied by R&D as the manufactures' instruction.

### Flow cytometry analysis of apoptosis with Annexin V–FITC/PI staining

Apoptosis assay was performed by flow cytometry according to the manufacturer's protocol (BD BioScience, San Jose, USA). Briefly, cells from 70% confluent plate were treated with different concentration of LL-37 polypeptide. The harvested cells were washed twice with cold PBS, and suspended in binding buffer. Aliquots of 100  $\mu$ l suspension ( $1 \times 10^5$  cells) was incubated with 5  $\mu$ l Annexin V-FITC and 5  $\mu$ l PI for 15 min at room temperature in the dark. Cell suspension was added with 400  $\mu$ l of binding buffer, gently vortexed, and analyzed within 1 h by flow cytometry (Becton–Dickinson, Oxford, UK). Apoptotic cells were expressed by percentage of Annexin V-FITC-positive cells of total gated cells.

### Statistical analysis

All data were analyzed using the SPSS 16.0 software. The Kruskal–Wallis nonparametric H test and Mann–Whitney nonparametric U test were used for comparison of LL-37 in induced sputum between groups. Correlations between LL-37 and pulmonary function parameters, SGRQ scores and 6MWD were assessed by Spearman's rank correlation test. Independent sample t-test and ANOVA were used to compare quantitative variables. Chi-square test and Fisher's exact test were used to compare categorical variables. A P-value <0.05 was considered significant.

## Results

### Clinical data

COPD patients were divided into I + II COPD and III + IV COPD according to FEV<sub>1</sub>%. All subjects were well matched for age and gender. Smoking histories were not significantly different between healthy smokers and COPD patients. FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC and 6MWD were significantly lower in COPD patients compared with healthy non-smokers and healthy smokers ( $p < 0.05$ , Table 1). SGRQ scores were significantly higher in COPD patients than in the other two groups ( $p < 0.05$ , Table 1).

**Table 1** Clinical data for the subjects included for serum and sputum detection.

	Healthy non-smokers	Healthy smokers	I + II COPD	III + IV COPD
<i>n</i>	18	14	13	15
Age (years)	63.1 ± 7.4	60.1 ± 9.8	61.8 ± 7.9	63.5 ± 9.2
Male/female	12/6	9/5	9/4	9/6
Smoking history (pack-years)	0	43.6 ± 23.7	42.8 ± 26.8	46.9 ± 27.4
FEV <sub>1</sub> %	90.4 ± 8.5	84.1 ± 7.8	57.1 ± 6.9	23.4 ± 5.8
FEV <sub>1</sub> /FVC	77.6 ± 6.8	74.9 ± 4.7	54.6 ± 7.6	34.1 ± 7.2
SGRQ scores	2.5 ± 1.5	3.5 ± 1.5	20.5 ± 8.5	45.7 ± 7.6
6MWD	539.5 ± 86.3	529.6 ± 68.1	456.2 ± 70.5	420.5 ± 87.5

Data are presented as mean ± SD.

### LL-37 levels in serum and induced sputum

To study the expression of LL-37 in serum and induced sputum, we performed ELISA analysis in 28 COPD patients, 18 healthy non-smokers and 14 healthy smokers. As shown in Fig. 1, induced sputum LL-37 levels were significant higher in I + II COPD and III + IV COPD patients compared with healthy non-smokers and healthy smokers (I + II COPD patients vs III + IV COPD patients vs healthy non-smokers vs healthy smokers Mean ± SD 4.37 ± 4.19 ng/ml, 7.99 ± 4.01 ng/ml, 0.74 ± 0.61 ng/ml, 2.64 ± 1.93 ng/ml,  $p < 0.05$ ). The expression of LL-37 was elevated in III + IV COPD patients compared to I + II COPD patients ( $p < 0.05$ ), LL-37 levels were significant higher in healthy smokers than healthy non smoker while there were no statistically significant differences in serum LL-37 levels between these four groups (I + II COPD patients vs III + IV COPD patients vs healthy non-smokers vs healthy smokers Mean ± SD 0.46 ± 0.14 ng/ml, 0.49 ± 0.14 ng/ml, 0.47 ± 0.18 ng/ml, 0.51 ± 0.16 ng/ml).

### Correlation analysis

To examine the potential role of LL-37 in disease activity or progression, LL-37 levels were correlated with pulmonary function as determined by FEV<sub>1</sub>%, SGRQ scores and 6MWD. Sputum LL-37 levels in COPD patients showed inverse correlation with FEV<sub>1</sub>% ( $r = -0.32$ ,  $P = 0.01$ ) and 6MWD

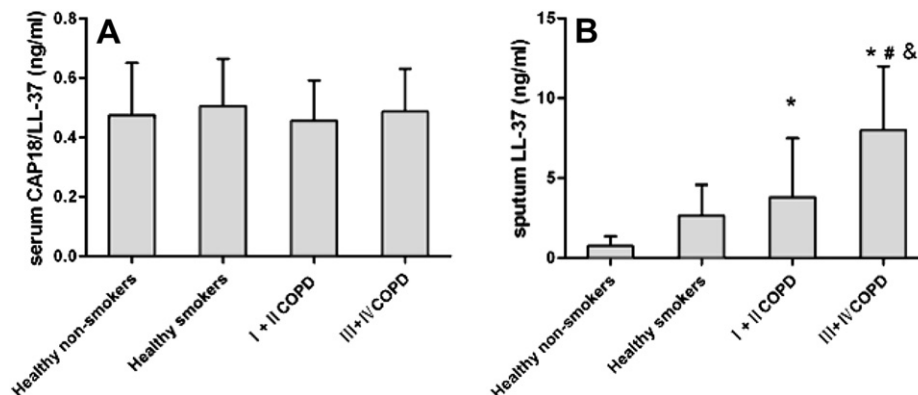
( $r = -0.31$ ,  $P = 0.01$ ), and positive correlation with SGRQ scores ( $r = 0.35$ ,  $P = 0.01$ ).

### Expressions of LL-37 in lung tissue

The clinical characteristics of the subjects for Immunohistochemical staining are shown in Table 2. All subjects were well matched for age and gender. Smoking histories, FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC% were not significantly different between COPD patients and control. LL-37 in lung tissue was mainly expressed on bronchial epithelial cell, alveolar epithelial cell, inflammatory cell (including neutrophils, monocyte-macrophage and lymphocyte) and fibroblast. The expressing intensity of positive cell in both airway area and pulmonary alveoli area in COPD group was significantly higher than that in control group (Fig. 2).

### Cytotoxicity induced by CSE and LPS

To examine the toxic effects of CSE and LPS on cell viability, BEP2D and A549 cells were incubated with different concentration of CSE or LPS for 6, 12, 24, 48 h. As shown in Fig. 3, cell viability decline in both concentration- and time-dependent manners. According to these results, we selected 0.02 cig/ml, 0.04 cig/ml, 0.06 cig/ml of CSE and 10 µg/ml, 20 µg/ml, 30 µg/ml of LPS in the subsequent experiments.



**Figure 1** Concentrations of LL-37 in serum (A) and induced sputum (B) of healthy non-smokers, healthy smokers, and COPD patients. Data were analyzed using one-way analysis of variance or nonparametric tests. \* $p < 0.05$  compared with healthy non-smokers; # $p < 0.05$  compared with healthy smokers; &  $p < 0.05$  compared with I + II COPD patients.



**Table 2** Clinical data for the subjects included for immunohistochemical staining.

	<i>n</i>	Age	Male/female	Smoking history (pack-years)	FEV <sub>1</sub> %	FEV <sub>1</sub> /FVC%
Control	10	56.4 ± 7.9	6/4	54.0 ± 35.8	110.4 ± 17.3%	82.6 ± 8.3%
COPD	9	60.1 ± 8.6	5/4	53.6 ± 35.2	61.63 ± 15.9%	56.3 ± 10.5%

Data are presented as mean ± SD.

### Effect of CSE or LPS on the expression of LL-37

To investigate whether CSE and LPS can induce LL-37 expression, we assessed the levels of LL-37 in the protein levels through immunofluorescence staining with Confocal Laser Scanning Microscope (CLSM). The results of CLSM showed that expression of LL-37 was gradually increased after incubated 24 h with 0.02 cig/ml, 0.04 cig/ml, 0.06 cig/ml of CSE or 10 µg/ml, 20 µg/ml, 30 µg/ml of LPS (Fig. 4).

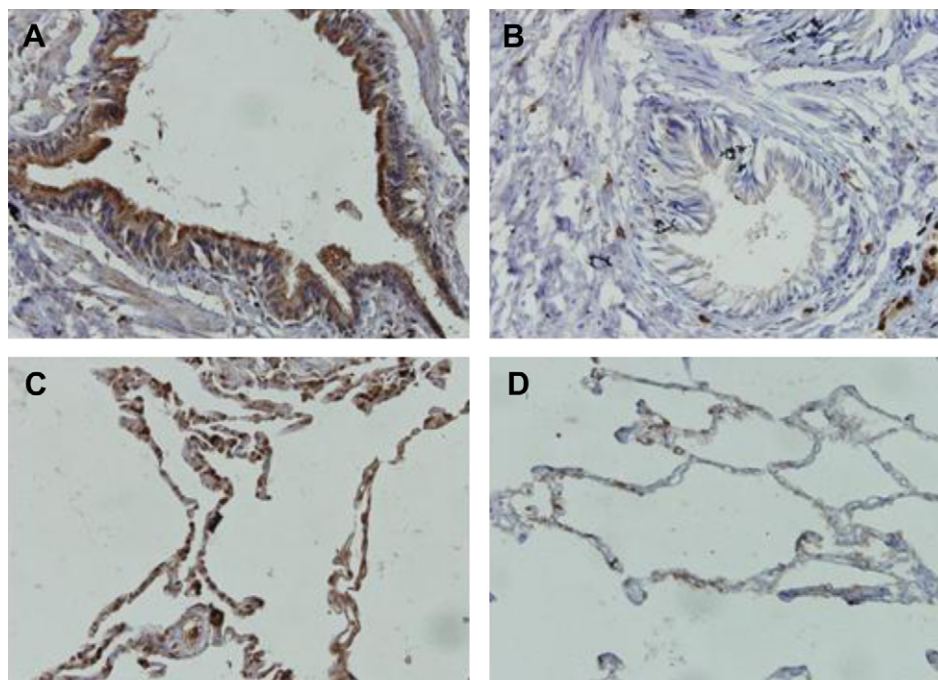
### Effects of LL-37 synthesis polypeptide on the release of IL-8 and TNF-α of BEP2D and A549

To explore the role of LL-37 synthesis polypeptide on inflammatory factors releasing, we assessed the levels of IL-8 and TNF-α in supernatants of BEP2D and A549 after

incubated with different concentration of LL-37 synthesis polypeptide by ELISA. As shown in Fig. 5, IL-8 were increased gradually in the supernatants of BEP2D and A549 after incubated with 10 µg/ml of LL-37 synthesis polypeptide while the level of TNF-α was not effected by LL-37 synthesis polypeptide.

### Analysis of LL-37-induced apoptosis by flow cytometry

Using fluorescent prone Annexin V–FITC and PI, apoptotic and necrotic cells were determined by flow cytometry analysis. The scatter plot of Annexin V–FITC and PI showed that LL-37 synthesis polypeptide can induce apoptosis of BEP2D and A549, showing dose and time-dependent effect (Fig. 6).



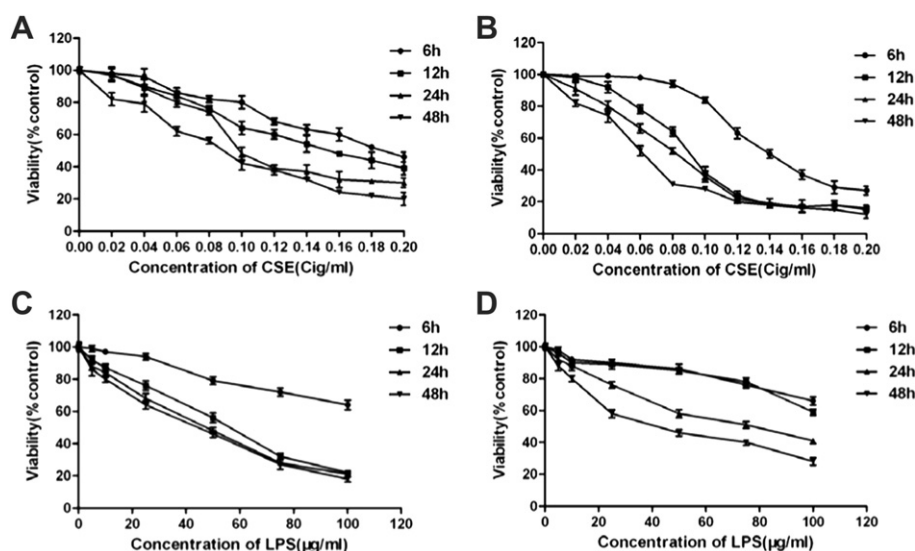
Expression of LL-37 in lung tissue of COPD and control group.

(Average absorbance, *n*=19,  $\bar{x} \pm s$ )

	<i>n</i>	Airway district	Pulmonary alveoli district
Control	10	0.122 ± 0.015	0.132 ± 0.021
COPD	9	0.261 ± 0.024 **	0.190 ± 0.017 **

\*\* *P* < 0.01 compared with control group.

**Figure 2** Representative light photomicrographs of immunostaining for LL-37 (brown staining) in small airway areas (A, B) and alveolar areas (C, D) in lung tissue from COPD patient (A, C) and control (B, D).



**Figure 3** Effect of CSE and LPS on the viability of BEP2D and A549. A. Viability of BEP2D cells after treated with the indicated doses of CSE for 6 h, 12 h, 24 h, 48 h. B. Viability of A549 cells after treated with the indicated doses of CSE for 6 h, 12 h, 24 h, 48 h. C. Viability of BEP2D cells after treated with the indicated doses of LPS for 6 h, 12 h, 24 h, 48 h. D. Viability of A549 cells after treated with the indicated doses of LPS for 6 h, 12 h, 24 h, 48 h.

## Discussion

LL-37, the only member of cathelicidins' family found in humans, was isolated from human bone marrow in 1995.<sup>25–27</sup> LL-37 is produced by neutrophils, macrophages and respiratory epithelial cells. The antimicrobial domain is released by cleavage by proteases and this domain is termed LL-37. In the present study, in order to explore the possible role of LL-37 in the pathogenesis of COPD, we thus first examined the expression of LL-37 in induced sputum and lung tissue of COPD patients and then explored the possible mechanism of LL-37 in COPD *in vitro*.

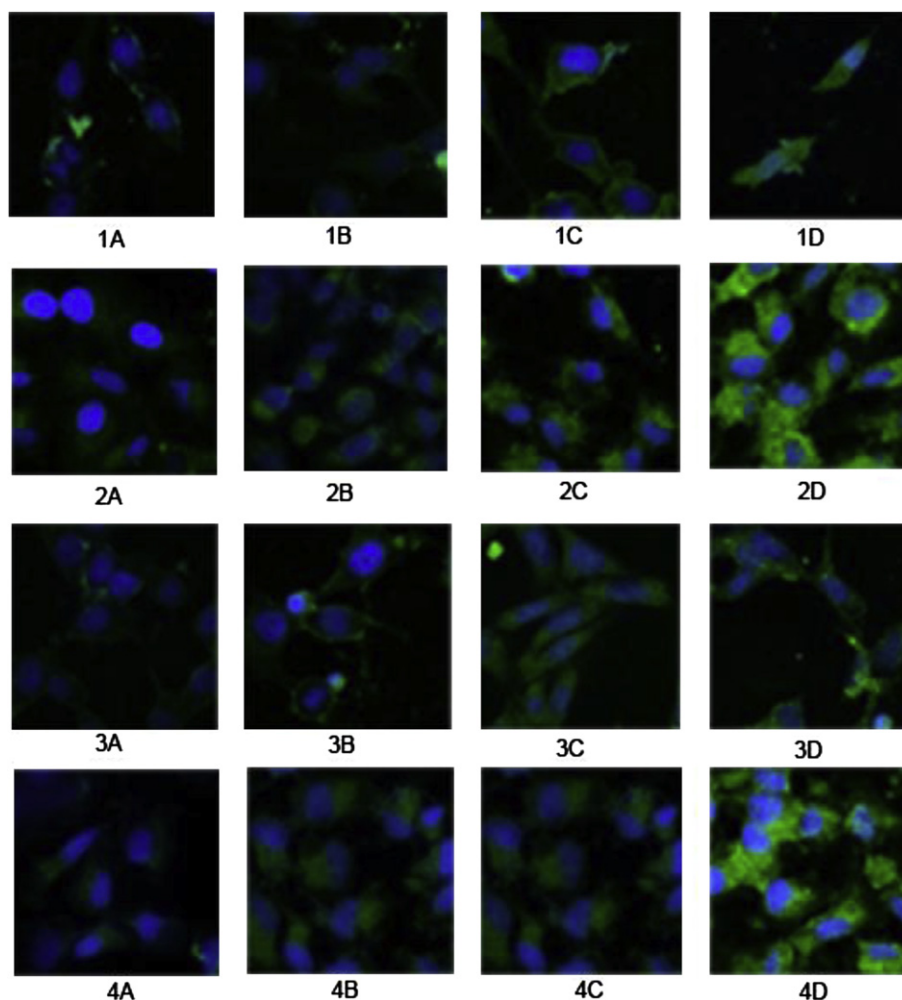
We initially found that LL-37 levels in induced sputum in COPD patients were significantly higher than control subjects and there were inverse correlations between sputum LL-37 levels and FEV<sub>1</sub>% and 6MWD, and a positive correlation between sputum LL-37 levels and SGRQ scores. Another research published in 2009 which similar to ours also showed high Cathelicidin LL-37 levels in induced sputum from farmers with COPD.<sup>28</sup> These results suggested a potential role for LL-37 in COPD.

The differences in sputum levels may have resulted from local production due to an airway inflammatory response. However, why the levels of LL-37 were raised up in stable COPD patients while there was no significant infection in these people? In order to explore the source of LL-37, we examine the expression of LL-37 in lung tissues of COPD patients with immunohistochemistry. The results showed that LL-37 was mainly localized in bronchial epithelial cell, alveolar epithelial cell, inflammatory cell and fibroblast. The expressing intensity of positive cell in COPD group was significantly higher than that in control group. This result was consistent with the previous reports that LL-37 was expressed on respiratory epithelium and neutrophils.<sup>29</sup> Therefore, these results explained the abnormal increase of LL-37 in induced sputum in COPD patients was not simple

because of the high rate of neutrophils, but an immune response resisting outside stimulation that many cells participated. Bronchial epithelial cell and alveolar epithelial cell, as important components of innate immune system, play an important role in these processes which then strongly suggesting us LL-37 may be involved in the pathogenesis of COPD. Lung tissues come from COPD patients receiving operation because of lung cancer, lung bullae, lung cyst. Lung cancer was not excluded because of the following two reasons. First, patients receiving operation because of benign lesions are limited. Second, there was just one paper showed that LL-37 is expressed mostly in adenocarcinoma and squamous cell carcinoma while the expression in adjacent lung tissue is not reported.<sup>30</sup>

What role does LL-37 play in the development and progression of COPD? What is the exact mechanism? We subsequently carried out some *in vitro* study. Cigarette smoking and infection were two main generally accepted causes of COPD. Bronchial epithelial cell and alveolar epithelial cell, as important component of respiratory immune system, are widespread and large in quantity. They are the first barrier existing external stimulation and exposed noxious particles or gases in the pathogenic process of COPD. So *in vitro* we selected CSE and LPS. The results of immunofluorescence staining showed that exposure to CSE or LPS can enhance the expression of LL-37. This results together with that in induced sputum and lung tissue showed that LL-37 may be involved in the pathogenic process of COPD.

What is the effect of the elevated LL-37 on lung cells? We investigated whether LL-37 could directly influence cytokines secretion. IL-8 and TNF- $\alpha$  are two important generally accepted inflammatory markers in COPD, and their concentration in induced sputum is considered to be good biomarkers for disease activity and progression in COPD.<sup>31</sup> Our results suggested that the secretion of IL-8 in



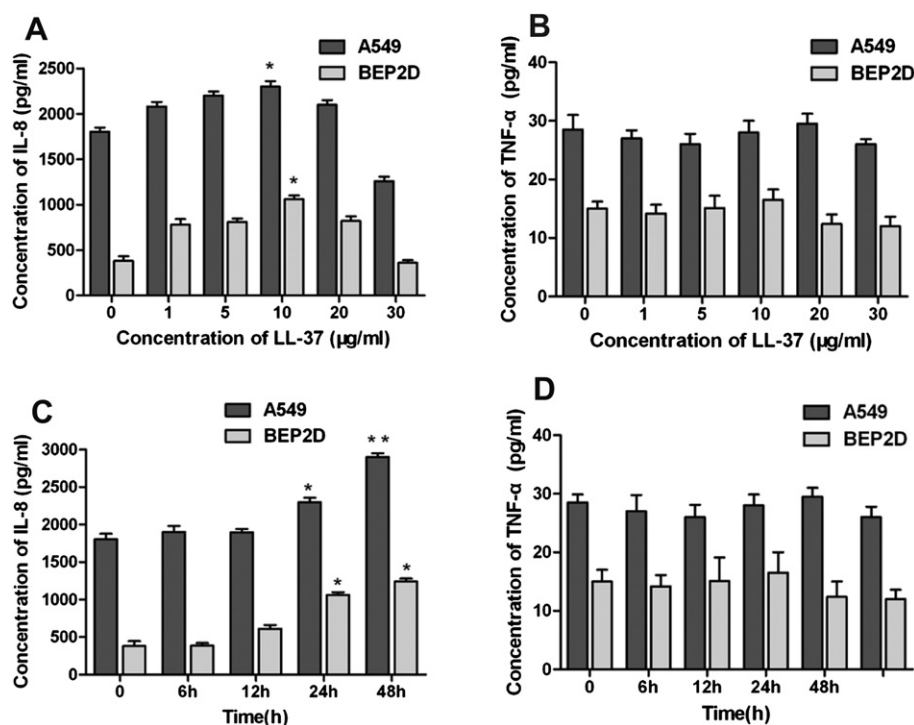
**Figure 4** Effect of CSE or LPS on the expression of LL-37. (1A) Expression of LL-37 on BEP2D before incubated with CSE. (1B)(1C)(1D) Expression of LL-37 on BEP2D after incubated 24 h with 0.02, 0.04, 0.06 cig/ml of CSE. (2A) Expression of LL-37 on A549 before incubated with CSE. (2B)(2C)(2D) Expression of LL-37 on A549 after incubated 24 h with 0.02, 0.04, 0.06 cig/ml of CSE. (3A) Expression of LL-37 on BEP2D before incubated with LPS. (3B) (3C)(3D) Expression of LL-37 on BEP2D after incubated 24 h with 10, 20, 30  $\mu\text{g/ml}$  of LPS. (4A) Expression of LL-37 on A549 before incubated with LPS. (4B)(4C)(4D) Expression of LL-37 on A549 after incubated 24 h with 10, 20, 30  $\mu\text{g/ml}$  of LPS.

bronchial epithelial cell and alveolar epithelial cell was induced after exposure to LL-37 synthesis polypeptide while the secretion of  $\text{TNF-}\alpha$  was not effected by LL-37 synthesis polypeptide. Scotts' gene array studies indicated that LL-37 directly up-regulates 29 genes which encoding chemokines and chemokine receptors.<sup>7</sup> Consistent with this, LL-37 up-regulated the expression of chemokines in several different cells which included macrophages and the mouse lung (monocyte chemoattractant protein 1), human A549 epithelial cells (IL-8), and whole human blood (monocyte chemoattractant protein 1 and IL-8).<sup>7</sup> According to the literature and our results, we speculate that LL-37 may induce respiratory epithelium cell to produce IL-8, which could in turn recruit additional immune cells to the sites of airway. This made the inflammation of COPD become persistent and irreversible chronic inflammation state which can be still exist without external stimulation.

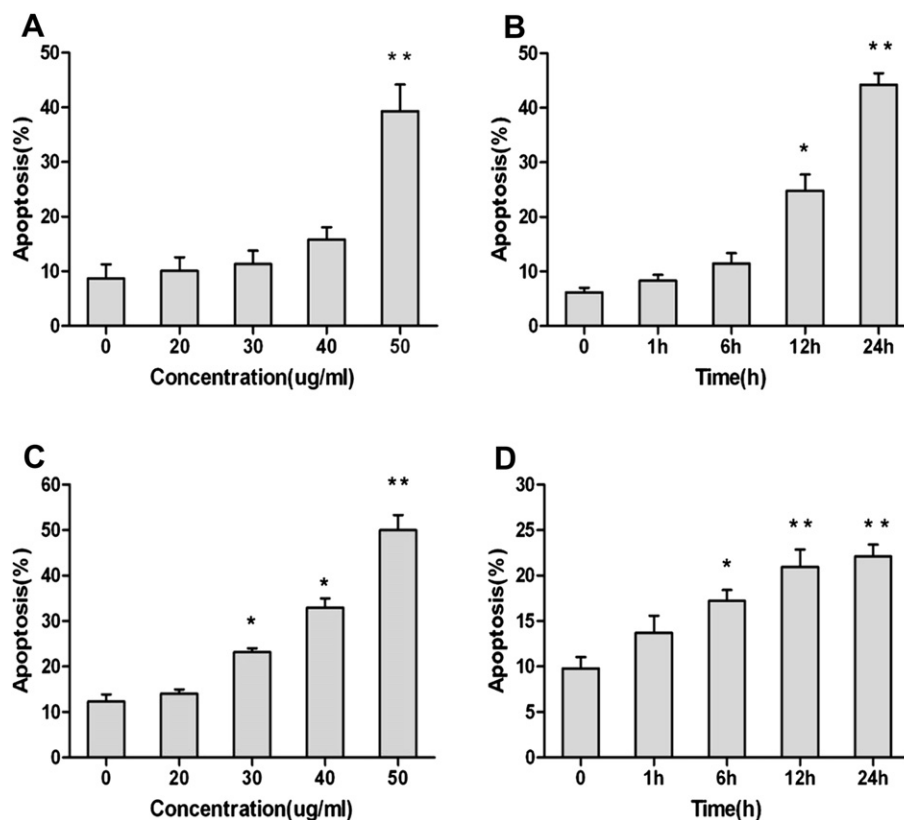
Research shows that lung epithelium is the primary site of lung damage in various lung diseases. Epithelial cell

apoptosis has been considered to be initial event and is followed by remodeling processes. More and more evidences suggest that apoptosis is involved in initial injury and defective repair in COPD.<sup>32,33</sup> For example, endothelial and epithelial cell apoptosis have been implicated as one of important mechanisms of pulmonary emphysema. Intratracheal injection of activated caspase-3 induces epithelial cell apoptosis, enhances elastolytic activity, and subsequently induces emphysematous changes in mice.<sup>34–36</sup> Our results showed that LL-37 synthesis polypeptide can induced apoptosis of bronchial epithelial cell and alveolar epithelial cell, which might be a possible mechanism of LL-37 involved in the development of COPD.

In conclusion, increased induced sputum levels of LL-37 in COPD patients were associated with airflow limitation, health status and exercise tolerance and expressing intensity of LL-37 in both airway area and pulmonary alveoli area in COPD group significantly increased compared with control group. These results suggested that LL-37 may be a valid biomarker for disease activity and progression in



**Figure 5** Effects of LL-37 synthesis polypeptide on the release of IL-8 and TNF- $\alpha$  of BEP2D and A549. A IL-8 in supernatant of BEP2D and A549 cells after treated with the indicated doses of LL-37 for 24 h. B TNF- $\alpha$  in supernatant of BEP2D and A549 cells after treated with the indicated doses of LL-37 for 24 h. C IL-8 in supernatant of BEP2D and A549 cells after treated with 20  $\mu$ g/ml of LL-37 for 6 h, 12 h, 24 h, 48 h. D TNF- $\alpha$  in supernatant of BEP2D and A549 cells after treated with 20  $\mu$ g/ml of LL-37 for 6 h, 12 h, 24 h, 48 h.



**Figure 6** Effect of LL-37 synthesis polypeptide on apoptosis of BEP2D and A549. A BEP2D cells were treated with the indicated doses of LL-37 for 24 h. B BEP2D cells were treated with 50  $\mu$ g/ml of LL-37 for 1 h, 6 h, 12 h, 24 h. C A549 cells were treated with the indicated doses of LL-37 for 24 h. D A549 cells were treated with 50  $\mu$ g/ml of LL-37 for 1 h, 6 h, 12 h, 24 h.



COPD. Through stimulation by CSE and LPS, the expression of LL-37 was increased in bronchial epithelial cell and alveolar epithelial cell. LL-37 synthesis polypeptide can induce apoptosis of bronchial epithelial cell and alveolar epithelial cell and promote the releasing of inflammatory factor, suggesting that LL-37 may play important role in the pathogenesis of COPD.

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## Conflict of interest

None of the authors has any conflict of interest related to this manuscript.

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